STABILITY TESTS OF ALKYNYLGOLD(I)(NHC) COMPLEXES BY HPLC-DAD-MS

Henrik Hoffmeister and Ingo Ott

Institute of Medicinal and Pharmaceutical Chemistry; Technische Universität Braunschweig, Beethovenstraße 55, 38106 Braunschweig, Germany; henrik.hoffmeister@tu-bs.de

ABSTRACT

Gold organometallic compounds have been extensively investigated as potential anticancer metallodrugs and have shown a high potential regarding antiproliferative effects [Hickey, 2008; Andermark, 2016; Meyer, 2012; Schmidt, 2019]. Enhanced stability is a driving argument for the design of gold complexes with *N*-heterocyclic carbene (NHC) ligands. The more surprising is the lack of methods of pharmaceutical analytics for their stability and solution chemistry. Such analytical methods are important key elements for future metabolomic investigations and will help to ensure a better understanding of the biological behavior of the complexes [Kostiainen, 2003].

We selected complexes of the type of alkynylgold(I)(NHC) for detailed stability studies by HPLC-DAD-MS, in comparison to the well-known antirheumatic drug Auranofin. A RP-based chromatographic method was established to separate possible degradation products of alkynylgold(I)(NHC) complexes. The stability studies were performed at 37°C over 24h using dimethylformamide (DMF), dimethyl sulfoxide (DMSO), water and Dulbecco`s modified eagle medium (DMEM) solutions of each compound. Furthermore, interaction experiments of alkynylgold(I)(NHC) complexes with acetylcysteine are under way with the same set-up as the stability tests [Albert 2012].

ESI (+) and (-) ionisation with a quadrupole analyser was used for mass spectrometry. The first results indicate that alkynylgold(I)(NHC) complexes are stable in the analysed solvents with no significant changes in their AUCs [Fig 2].

INTRODUCTION

N-heterocyclic carbene (NHC) ligands with their strong σ-donating character can be coordinated well to several transition metals. Specifically, gold(I) complexes have been extensively investigated and have shown a high potential regarding antiproliferative effects [Hickey, 2008; Andermark, 2016; Meyer, 2012; Schmidt, 2019]. The enhanced stability has been a driving argument for the design of metal-NHC based drugs. However, extended studies on their solution chemistry and stability have not been frequently performed and aspects of pharmaceutical analytics have not been considered sufficiently. We selected complexes of the type alkynylgold(I)(NHC) (Fig. 1) for detailed stability studies by HPLC-DAD-MS, in comparison to the well-known antirheumatic agent Auranofin.

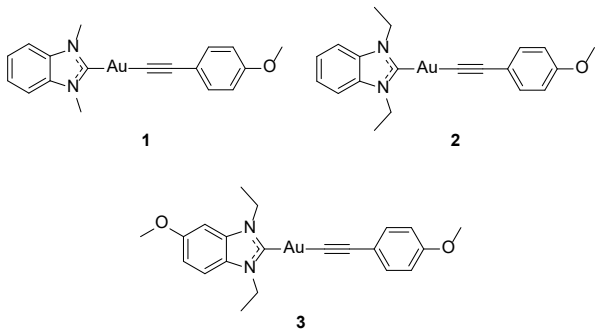

Figure 1: Structures of selected alkynylgold(I)(NHC) complexes

On top of that an interaction experiment of alkynylgold(I)(NHC) complexes with acetylcysteine is under way with the same set-up as the stability tests.

EXPERIMENTAL

The stability studies were performed in four different solvents (DMF, dimethyl sulfoxide (DMSO), water and

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Dulbecco`s modified eagle medium (DMEM)) and were carried out with an established HPLC method. The first step was to prepare a DMF stock solution, which was diluted with the respective solvent. For the stability tests in water and DMEM the organic solvent (DMF) amount had to be increased to 50% of the total volume because of the poor water solubility properties of the compounds. After transferring the solutions into the HPLC vials the incubation at 37°C was done in the auto-sampler unit of the HPLC. The time-dependent injection could be automated. For this study the injection was done after 0h, 4h, 8h, 12h and 24h.

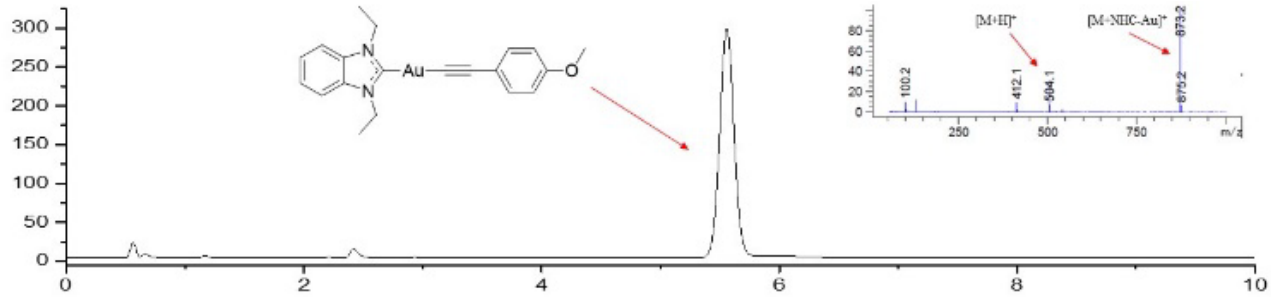

Figure 2: Chromatogram of a selected complex in DMEM after 24h. The insert shows the ESI (+) mass spectrum of the main peak.

The interaction experiment was performed in three different solvents: water, phosphate-buffered saline (PBS), 2-amino-2-(hydroxymethyl)propane-1,3-diol (Tris) buffer. Initially acetylcysteine and the alkynylgold(I)(NHC) complexes were characterized in the solvents mentioned above over a time period of 24 hours. After the characterization an equimolar solution of acetylcysteine and each alkynylgold(I)(NHC) complex were prepared and incubated together at 37°C over 24 hours in the autosampler unit. The injections were automated and measured under the same conditions as the stability tests.

DISCUSSION AND CONCLUSIONS

Over the exposure time no significant peak changes were observed. Moreover, the peak areas remained unaltered over 24 hours.

In the case of the interaction experiment a different behavior could be observed. In water the peak for the alkynylgold(I)(NHC) complex disappeared almost completely after 2h and a new peak with a much shorter retention time occurred in the chromatogram, which could be identified as $Au(NHC)_2^+$ by means of MS detection. A similar behavior can be observed in PBS and Tris buffer. In case of PBS the decrease of the main peak is much lower and even after 24h a significant amount of alkynylgold(I)(NHC) complex is still present in the sample. Almost the same behavior can be observed in Tris buffer, however, the effect is much less developed.

NOMENCLATURE

AUC	area und curve
DAD	diode array detector
DMEM	Dulbecco`s modified eagle medium
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
ESI	electrospray ionisation
HPLC	high performance liquid chromatography
MS	mass spectrometry
NHC	*N*-heterocyclic carbene
PBS	phosphate-buffered saline
Tris	2-Amino-2-(hydroxymethyl)propane-1,3-diol

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